

# BIOSCAN: Illuminating Biodiversity

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international  
BARCODE  
OF LIFE



## Audience Q&A with Dan Janzen and Winnie Hallwachs

*Nine Malaise Traps in Three Costa Rican Forest Hectares Catch ~40K Mostly Undescribed Species of Insects Among 1,521,762+ Specimens in Seven Years:* **NOW WHAT?**

**What sort of results can you expect to get from all this data?**

*Will Bayfield-Farrell*

Not meant to be snippy or snarky, the reply is “What do you expect a first grade student to do with learning to read and being introduced to an open library of the world’s literature (and internet)?” I expect that student to be literate all their life, with all the heterogenous implications of that, and as a consequence, willing to welcome the literature and literacy itself. Same for BioLiteracy based on being able read wild (or domesticated) biodiversity (what really was in your hamburger?), no matter what form or place. A literate person can read a STOP sign, the Magna Carta, The Merchant of Venice in 10<sup>th</sup> grade high school, and all of them when retired in assisted living, and even several languages.

In most brief form:

1. The initiation (already) of a DNA barcode reference platform, public and vouchered, for Costa Rica as an example of what a tropical species-rich country can do with its surviving natural capital rather than turn it yet more into industrial agroscape feeding overweight internationals and nationals.
2. Greatly increased understanding by all sectors of national and international of what actual biodiversity still exists, both through absorption by the “science” guilds and the village.
3. A major increase in financial integration of biodevelopment into today’s ever-more-simplified lives driven in large part by homogenizing, dumbing down, industrialization, pandemics, the omnipresent human genome and its derived cultures, etc.
4. A more accurate understanding of the living world and what it actually is.
5. More human visible conflicts as the various forms of serfdom come to be recognized for what they are.
6. More survival of the remaining remnants of tropical wild biodiversities.

And more...

**Are you planning to use barcoding technology in something else in Costa Rica?**

**And what is the state of the project in the country right now?**

*Eduardo Rodríguez*

For the something else, we are simply barcoding-money processing limited. The results will be available for others to use. The sweat equity is there to get the samples of plants, vertebrates, nematodes, mites, litter fauna, etc. The big cost is the actual CBG cost per sample (per bug) and in parallel to support the classical taxonomic framework onto which to hang the barcoding results and build upward with the two integrated. We are trying to cover both simultaneously with the pitifully small amount of funding that we can raise, and more disastrous in the COVID years, but by trying to cover both, we can do less of both. We have to currently raise about \$200k/year as the ACG contribution to the classical taxosphere, in addition to the huge amount of sweat equity they offer, plus whatever we can find as our support for the Guelph operation in \$\$ and sweat equity.

Current state in Costa Rica right now is that any new pilot projects have to be funded by the project itself (e.g. separate small grants for a high school year, about \$75k for two traps for one school, or a national park) or fully self-funded for a commercial operation (e.g., Dole Pineapple plantations, luxury rural hotels, ecolodges). At present, all we have is the funds for attempting to keep the inventory of ACG in motion, not expanding. IF BioAlfa does not receive major funding now or in the beginning of 2022 it will have to go on hold, restricting its national reach until funding appears or it dies entirely as simply unrealistic, just demonstrating what can be done with one large national park, IF you, they, want to. At this moment BioAlfa has about 2.5 million frozen specimens from sampling, waiting for funding to continue forward, like tomorrow. With another year of real start up, that number could be doubled. The national sweat equity is ready and waiting, both really and biopolitically. Our pitifully small staff, mostly funded by other processes, is exhausted.

**How does BioAlfa's experience change the previous taxonomic projections for Costa Rica's biodiversity (species richness) as a whole?**

*Gustavo Induni [SINAC]*

Way before pre-barcoding, in the mid-1960's when preparing to do a total biodiversity inventory of ACG, we estimated Costa Rica to contain about 500,000 multicellular terrestrial and freshwater species. This estimate came from interviewing 60+ very high level global taxonomic experts for their opinion for Costa Rica and the rest of the world. That is the basis for the 4% Costa Rica and 4% North America estimates. Following the first 10 years of DNA barcoding hundreds of thousands of ACG adult caterpillars and their wasp and fly parasites, of about 7000 species at that time we doubled that estimate, based on the cryptic species richness being revealed. This includes new species among believed-to-be-identified (the record so far is 34 species under one name (*Apanteles leucostigmus*, Braconidae)) and the enormous number of (mostly new) species appearing as a consequence of thorough year-round and cross-ecosystem inventory by parataxonomists. Adding more recently the Malaise traps where everything is barcoded, rather than just your favorite taxon, has now made us realize that the 1,000,000 species estimate for Costa Rica is certainly way too low. 1,500,000 species would be reasonable, though when fungi and nematodes and mites and spiders, and lower plants are added in (lichens etc.) that may still be too low - to say nothing of the microbes, viruses and protista.

However, it is clear to us the national estimates for other tropical countries are grossly too low. Colombia advertised that they had 10,000 insect species along with their well-counted vertebrates. I would guess them to be 8,000,000 species of invertebrates, fungi and other multicellulars. The problem always has been that a country has tended to view what is in their museums as representing their species-richness, rather than tackling their own real living world.

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**What is next with the thousands of undescribed species?**

*Eugenie Phillips-Rodríguez*

There is what IS next and what we would HOPE will happen. We prefer to focus on the latter. Barcoding creates two parallel lineages of action, hopefully integrated, intertwined and reinforcing each other. What we see is pragmatically supportable and desirable by the society that pays the bill, is that without worrying about getting formal "scientific names" on the species before they can be "used", the barcodes and BINs can be used as proxy names for many kinds of use - bioprospecting, legislation, novel ideas, biomonitoring in farming and medicine (a kind of farming), aesthetics, recreation, education as to what biodiversity actually does, etc. I do not have to know the scientific name of coffee to predict and discuss that its prime production habitat is sliding up Costa Rican mountains with the heating of climate change. BUT, if discussing which species and varieties of "coffee" will be able to produce at lower elevations, we need scientific names (or agreed vulgar names) for those different gene pools for all the obvious reasons.

So, while barcoding can move biodiversity out of its doldrums for society, more classical morphology-based taxonomy can hope to be moved ahead in parallel, integrating what barcoding offers it, each feeding off the other, and each offering more goodies to the society that tolerates and supports (hope) both taxonomic biology and the organisms for which it has long been invented. This does however, require more funding from an already-taxed society, an already overcrowded society, to allow those who have the inclination to be taxonomists and taxonomy users (such as we are) to fully express those abilities and inclinations. We do not need to "train" more taxonomists, we need to fund more jobs for taxonomists, along with empowering them with new tools such as barcoding, both for its science and for its ability to show the value of identification and inferential taxonomy to all sectors.

So, we would like to see those thousands (literally) of new species not only in ACG but in all of Costa Rica and the tropics get names, but also to be treated as members of society while they are getting them. That means both processes need to become commonplace in budgets, just as are taxes, vaccines, literacy, and human languages.

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**Are you planning on including marine ecosystems as well?**

*Emma PT*

We of course would like to have BioAlfa expand to the Marine portion of Costa Rica, and it is technically and sweat equity biopolitically possible, but there are no funds except for a small amount now done as pilot project in the Sector Marino of ACG (43,000 ha). Winnie and I personally are familiar with marine biodiversity, but in no way sufficiently understanding of it to even begin to guide such an endeavor.

There ARE Costa Rican biologists who would be very good leaders there, but there truly are no funds available to fuel such an administratively and logistically complex action, to say nothing of the barcoding itself.

**How can I participate as a citizen scientist and send my samples?**

**How much would it cost?**

*Alvaro Figueroa*

If we are talking about samples from some other country, and that would be a direct negotiation between you and the Centre for Biodiversity Genomics AND taking into consideration the relevant legislation of your country vis a vis its own biodiversity samples (specimens and genomics). If we are talking Costa Rica, I can aim you at the place to start, but I would need to know the sort of objective, sort of taxa in mind, and the rest gets tailored by detail by CBG or BioAlfa, depending. The actual barcoding will currently cost you between \$1 to \$10/sample depending on things like how many, how and when captured/sampled, how old, what you expect as technical results. AND your barcodes become public domain through BOLD. If you allow BioAlfa to know your details, we may well be able to offer hints from our own experience since 2004, both about the Costa Rican situation and the Guelph very different in-house processing situation.

**Has the data from agricultural fields started to come in? Presumably there are other types of disturbances (chemical spraying) in that case?**

*Dan Bock*

That will be perhaps a month (?) from now. The results will guide company willingness to pay for more results, both with respect to the wild things adjacent to the agroscape and in it our ability to analyze that data will depend on both the barcodes, and the connections that we can make already between them and what standard morphologically-based taxonomy knows about both their taxonomic inferences and the actual species. Standard taxonomy already knows a crambid moth larva mines in pineapple stems and roots, but barcoding may alert us to it being two or more species than the believed one, and that may set off all sorts of interactions of within and between country agroscares. There is more to the interactions of wild biodiversity with its society than just a believed name – as the various mutants of COVID-19 display so well today. So, we see what happens, just as was the case with biomonitoring the geothermal development project. Just as we would love to see the taxosphere take on DNA barcoding as a high quality tool, agriculture could do the same. Medicine has been hoping on doing disease barcoding for decades already.

I failed to mention that in the DOLE pineapple case, BioAlfa has two traps associated with the organic fields and two with the conventional fields, simultaneously. They are analogues to the deep forest and forest edge in the case of the geothermal biomonitoring project.

**Do you think that Environmental Impact Assessment guys may be seriously interested to start using insect community data as a new/complimentary approach to traditional assessments?**

*Gustavo Induni [SINAC]*

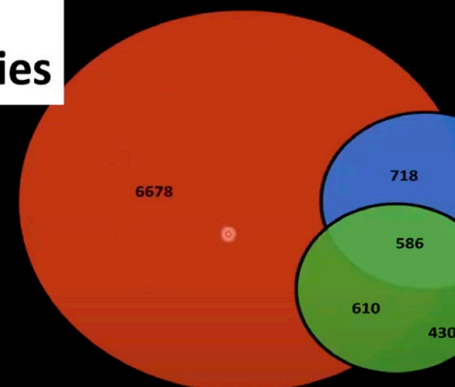
Yes, at first ICE (the National Electric company) was logically timid and neutral, but over two years of experience working with, on the ground, the ACG as interested in its own agenda (build the library and minimize (and know) the damage to the ACG margin (none)), ICE decided that yes, they had to comply with classical legal requirements (not damage endangered species, etc.) but also that this new method had very large potential for biomonitoring. We can direct you to the foresighted individuals in ICE if that is desired. They are your same Ministry, I add for outsiders. Many are coauthors of the resultant scientific-applied paper about the first year of PL12 barcoding, available at <https://doi.org/10.1139/gen-2020-0002>. The Japanese government, whose loan to Costa Rica paid to develop the geothermal sites at Pailas, made the same mental conversion, and paid the barcoding bill for the first year, yet were adamant about not funding “research”, but only “development”.

**When the existing frozen specimens at Guelph are barcoded, and the ICE and High School sites are activated and barcoded with sequences in BOLD, how will you scale the sort of Venn diagram analysis (Timestamp – 21:03) you presented from PL-12 and make them accessible to the world?**

*SIBT*

Just plain do it, and a LOT more analyses as well, concocted and designed so as to be maximally available to both the ivory tower science community and society at large. That presentation will be highly receptive to petitions from different sectors. It will all be on the web in both English and Spanish (and other languages if the petitioning society provides the resources to make it happen). For example, Japanese and Chinese for the ecotourism industry, or green industry desires, such as biomonitoring the geothermal site or industrial agriculture. However, just as has already happened once with PL12 (see <https://doi.org/10.1139/gen-2020-0002>). Because some sectors, e.g., the Ivory Tower silo does not absorb anything but certain published documents.

**PL12-3  
8,592  
species**



**Where are the insects stored?  
Can they be made available  
to specialists? What are the  
conditions of specimen loans?**

Wieslaw Krzeminski

These actual insects go by two basic routes. Those that are Malaise trapped go as weekly (per trap) bags to the CBG in ETOH and they do all the sorting, voucher coding, etc. After being barcoded, the insect carcass (if small and whole beast extracted, or a leg from a pinned specimen if larger) is stored in ETOH or on a pin (dry). If someone wants to do taxonomy with that particular specimen or all representatives of that taxon, you ask the CBG, they ask me as the Costa Rica representative if the CBG is allowed to send you the samples of the carcass along with its label information as a loan to be returned. Or we arrange beforehand that your museum becomes the recipient of a permanent loan = gift that your museum retains, does not send onward. In other words, the “deal” is fine-tuned to the situation. There are very few of these in motion, mostly because we have been approached by very few hardcore taxonomists for the loan or donation of specimens as part of their taxonomic work on these specimens. Wish we had a lot more taxonomists, AND in that case, some funding to cover their costs to us and the CBG.

The other route is the reared specimens. Those are treated individually. Each caterpillar found by the parataxonomists (PTs) in the field is at that times given its unique voucher code (really for the record of finding it) by the same PTs, and databased (one record per caterpillar), sometimes photographed (separate database), reared to an adult or parasite, frozen and passed to me, and I double check their field ID against what I see. They did 30-40,000 per year pre-decline, and now it is 15-20k records. I decide to a) discard, b) keep to mount and barcode, or c) save particular taxa for a collaborating taxonomist who has placed an order for more of whatever. The parasites in ETOH (one tube per caterpillar) go direct to Philadelphia to be de-legged, with the leg going into a lysis plate for barcoding (several thousands per year). Bigger parasites and Tachinidae go on pins and oven dried and treated the same way. The de-legged parasite vouchers end up in the Canadian National Collection or Smithsonian, as permanent deposits. The adult Lepidoptera to barcode are pinned and oven dried by the PTs in Santa Rosa, de-legged, photographed, etc. (these databases are joined through their unique voucher code for the specimen and its photographs and its primary record). Then brought to Philadelphia and distributed to some collaborating taxonomists but mostly deposited in the Smithsonian, and nearly all species also duplicated into the Museo Nacional de Costa Rica. Parataxonomists for the most part were farm workers, guards, etc. and learned all of this on the job from the original course in 1989-1992 and subsequent apprenticeships. It is a paid career.

Available to specialists? Most decidedly but each case is a collaboration between BioAlfa, Costa Rican legal regulations, biological reality, the collaborating taxonomist, CBG, and the international public museum where they will finally be deposited. BioAlfa keeps nothing but information, public domain, but there are “waystations” within the network. A taxonomist with interest in collaborating writes directly to us or to CBG, they ask us and we guide the process. A perfect example is Mike Sharkey's recent paper:

Sharkey MJ, Janzen DH, Hallwachs W, Chapman EG, Smith MA, Dapkey T, Brown A, Ratnasingham S, Naik S, Manjunath R, Perez K, Milton M, Hebert P, Shaw SR, Kittel RN, Solis MA, Metz MA, Goldstein PZ, Brown JW, Quicke DLJ, Van Achterberg C, Brown BV, Burns JM 2021. Minimalist revision and description of 403 new species in 11 subfamilies of Costa Rican braconid parasitoid wasps, including host records for 219 species. *ZooKeys* 1013: 1–665. <https://doi.org/10.3897/zookeys.1013.55600>

Other examples:

Fleming, A.J., Wood, D.M, Smith, M. A., Hallwachs,W., and Janzen, D. H. 2018. Revision of the Mesoamerican species of *Calolydella* Townsend (Diptera: Tachinidae) and descriptions of twenty-three new species reared from caterpillars in Area de Conservación Guanacaste, northwestern Costa Rica. *Biodiversity Data Journal* 5: <http://bdj.pensoft.net/article/11223>

Goldstein, P. Z., Janzen, D. H., Proshok, B., Dapkey, T. and Hallwachs, W. 2018. A review of *Lophomyra* Schaus, 1911 (Lepidoptera, Noctuidae): a new combination and re-descriptions of species newly associated with ferns (Polypodiaceae). *ZooKeys* 788:135-165.

Metz, M. A., Hallwachs, W. and Janzen, D. H. 2020. Four new gelechioid species to honor Costa Rica's conservation of wild biodiversity (Lepidoptera). *Zootaxa* 4810(1): 045-064. <https://doi.org/10.11646/zootaxa.4810.1.2>

Hansson, C., Hallwachs, W., and Janzen, D. H. 2021. New distributional, biological and taxonomic information on the genus *Eulophinusia* Girault (Hymenoptera: Eulophidae). *Zootaxa* 5047(3):370-376.

**I am currently working at the Natural History Museum, London. You mentioned sharing samples from your traps. Would it be possible to share some neotropical specimens with the entomological department here at the museum?**

*Will Bayfield-Farrell*

Most decidedly, but part of your reply is contained in our reply to Wieslaw Krzeminski above. The key bottom line would be which specific Natural History Museum taxonomists would like to take on portions of the barcoded specimens, for standard alpha taxonomy, as has been the case in the papers at the end of the Wieslaw Krzeminski reply above, and more. There are many forces working contra to established museum and university-based insect taxonomists fully adding the philosophy and tools in this talk to their careers. BioAlfa remains at this time at that frontier.

Please note that we are not taxonomists but rather very appreciative users of the products of the taxaphere's four centuries of dedicated and intense action. We are therefore use-driven in our relationships with conservation and biology. NHM's Ian Gauld (RIP) and CNC's Monty Wood (RIP) were very brave members of this frontier while being very hard-core taxonomists and very important to the germination and growth of the philosophy expressed here. We can only hope for more like them and support that reproduction.

**Are the barcoded specimens available for research?**

*Jennifer Girón*

Yes, but it depends on the kind of research. For taxonomy and phylogenetics, with barcodes or otherwise, all Costa Rica officially will want to know is the taxonomic details, destiny of the specimens, and other such pragmatics. Associated with each barcoded specimen at the CBG there is a frozen extract that contains the whole genome (to whatever degree the extraction was successful). That is also available for genomic work, but under explicit permit from the government CONAGEBIO (regulatory office for Costa Rican biodiversity) and that takes time, sometimes months.

Please remember that while the barcode is public domain, as well as the usual collection data, the specimen and therefore its genome is Costa Rican government property, and in effect, the CBG is custodian under a BMTA (Biological Material Transfer Agreement) that stipulates that CBG is obligated to inform Costa Rica of a desire for deeper exploration than the COI barcode or other markers.

I should also add that these new kinds of government regulations are ever evolving for tropical countries, and especially those that want to work internationally with their own natural capital. The Elgin Marbles problem is one that we all know, and BioAlfa has worked for decades with the Costa Rican government to not be in that situation.

**En la diapositiva que muestra los BIN's obtenidos para la familia Tachinidae, me surge la duda de como a partir de un numero mas reducido de BIN's se obtuvo una mayor cantidad de especies registradas. ¿A que se debe esta particularidad?**

*Oscar Prieto*

Yes, that is because some BINs contain several species, species that are discovered by groups WITHIN the BIN in the neighbour-joining tree. Upon looking at morphology, ecology or microgeography of those lumps, only shallow separated (less than about 2% different) we find that each of the lumps have different biologies.

In other words, the species has already split into three evolving units (sympatric) but their traits have become distinctive before their barcodes have. There is no particular reason to which sets of traits should evolve faster than the other. The outcome is that when we have thoroughly analysed a huge batch of specimens, all reared, and all barcoded, we tend to get about, for example, 900 BINs containing 1000 species. A ten percent increase.

So, if there are 2,000 BINS in a large sample from a Malaise trap session, for example (perhaps 25,000 specimens) a best guess is 2,200 real species in sample. This has proven to work across 3000+ species of Hymenoptera, about 900 species of Tachinidae, and about 11,500 species of Lepidoptera, to date, in ACG. My apologies for not making this clear in the presentation.

**Although the species count for reared braconids and tachinids was about twice as high as the Malaise traps, the sample size was lower for Malaise traps. Isn't that a bit surprising?**

*Paul Hebert*

Not really surprising. That is a standard empirical result with our inventories over the years. Our rearing sample is currently about 950,000 caterpillars from all parts of ACG. By the time ACG is thoroughly Malaise trapped in all its nooks and cranny ecosystems, we will re-examine the outcome.

The Malaise traps reported on here are just from the three major ecosystems, and as the PL12 results clearly show just the results of those 9 traps in PL12, even after 7 years the species accumulation curve per year is still rising with no sign of an asymptote, and I could care less what "curve smoothing" would like to anticipate. A curve calculated with each successive year would show an incorrect anticipated asymptote.

**Can you recommend any collection method which can improve sampling using Malaise traps to collect a broader part of the community?**

*Piotr Gadawski*

Yes, “old-time” entomologists, as I am, are walking libraries of such methods, and many could be applied along with the Malaise traps, yellow pan traps, etc. But they all come with the large lab cost of real barcoding for yet more samples (wonderful, but who pays the bills) and a few admin field operations costs.

And in the tropics, they also come with their own dose of biopolitics. We have right now more than a million specimens sitting in freezers, already collected by other methods, biopolitically approved, but cannot move ahead for lack of budget.

**Have you considered to launch some kind of crowdfunding to support the barcode sequencing efforts?**

*Marko Nieminen*

We have thought of it many times, but the massive administrative task and compromises that would go with it are super daunting. It would be like financing a hospital, highway, the internet, by crowdfunding. To date we are depending on small grants for pilot projects (e.g. three small sources for the first rural high school with about 25 students, the self-financed Dole pineapple project) or a very few large ones (administratively very simple) from government or private persons or foundations.

**How do you see your scientific findings inputting into policy since species names are the currency of biodiversity policy? (e.g., Convention on Biological Diversity or national policy)**

*Adriana Radulovici*

YES, this is very hard for some, and BioAlfa and barcoding receive pushback from that sector. You grit your teeth and bear with it as a real part of the world, like rain and droughts. And you invest decades in bringing up to speed the political and other sectors that create those species-based structures. Costa Rican senior policymakers can be very reasonably proud of themselves for actually knowing what are species, what is DNA, what is bioprospecting, what is barcoding when explained, what are ecosystems, what is climate change and why, pesticide impacts, biological education, etc.

But we also have to remember that each of those sectors is ALSO tied in knots by obsolete legislation, international positions (such as the Kyoto Protocol), well-meaning interference by strangers to a society and its desires as well as needs, COVID, global globalizations, truly harmful individuals that get a firm grip on power, multinationals, etc. Working with the affected has helped BioAlfa processes enormously, rather than staying within our science silo.

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